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Running title; Dairy fat and cardiovascular risk factors

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26

Abstract

Objectives: to evaluate the impact of three specific ruminant (R) milk fats resulting from modification of the cow's diet on cardiovascular risk factors in healthy volunteers. R-milk fats were characterized by increased content in total *trans* fatty acids (R-TFA) and parallel decrease in saturated fatty acids (SFA).

Methods: 111 healthy, normolipemic men and women have been recruited for a monocentric, randomised, double-blind, and parallel intervention, 4-week controlled study. Volunteers consumed 3 experimental products (butter, dessert cream and cookies) made with one of the 3 specific milk fats (55 g fat/day). During the first week (run-in period), the subjects consumed on a daily basis dairy products containing 72% SFA/2.85% R-TFA (called "L0"). For the next 3 weeks of the study (intervention period), the first group continued to consume L0 products. The second group received dairy products containing 63.3% SFA/4.06% R-TFA (called "L4"), and the third group received dairy products containing 56.6% SFA/12.16% R-TFA (called "L9").

Results: plasma concentrations of HDL-cholesterol was not significantly altered by either diet (p = 0.38). Compared to L0 diet, L4 diet contributed to reduce LDL-cholesterol (-0.14±0.38 mmol/L, p= 0.04), total cholesterol (-0.13±0.50 mmol/L, p = 0.04), LDL-cholesterol/HDL-cholesterol (-0.14±0.36, p = 0.03) and total cholesterol/HDL-cholesterol (-0.18±0.44, p = 0.02).

Conclusion: different milk fat profiles can change cardiovascular plasma parameters in human healthy volunteers. A limited increase of the R-TFA/SFA ratio in dairy products is associated with an improvement in some cardiovascular risk factors. However, a further increase in R-TFA/SFA ratio has no additional benefit.

Keywords: Human Nutrition, Lipids, *trans* fatty acids, milk fat, cardiovascular risk factors, cholesterol.

Introduction

Over 2 million people in EU are dying from Cardiovascular disease (CVD) every year(European Heart Network, 2008). The subsequent cost is estimated to €192 billion /y including direct and indirect cost. Thus, the reduction of the number of death from CVD is a huge target which could be reached by a limiting exposure to CVD risk factors. In this respect, dietary fatty acids represent key factors having a significant impact on health, especially on CVD. Specific effects of clusters or isolated fatty acids have been extensively studied, with a particular attention paid to saturated (SFA) and *trans* (TFA) fatty acids (Ascherio et al., 1999; Gebauer et al., 2007; Hu et al., 1997; Katan et al., 1995). Reports from different health authorities and agencies recommend a reduction of SFA and TFA intake (Afssa, 2005; Scientific Panel on Dietetic Products, 2004; Stender and Dyerberg, 2003).

Two meta-analyses tabulating different intervention studies clearly stated that TFA are more deleterious than SFA, when considering fatty acids' impact on cardiovascular risk factors (Ascherio et al., 1999; Mensink et al., 2003). Consequently, the relationship between the consumption of dietary TFA and the increased risk of CVD has been clearly highlighted (Booker and Mann, 2008; Dalainas and Ioannou, 2008; Gebauer et al., 2006) . However, all these studies considered industrially produced TFA isomers (IP-TFA) resulting from partial hydrogenation of oils, but TFA are also present naturally in ruminant milk fat and meat (R-TFA). R-TFA and IP-TFA have different isomeric profiles. In IP-TFA, *trans*-9 18:1 (elaïdic acid) and *trans*-10 18:1 are the most important isomers whereas *trans*-11 18:1 (vaccenic acid) is the major R-TFA isomer (Stender and Dyerberg, 2004). The R-TFA term comprises total trans fatty acids (all the geometrical isomers of monounsaturated and polyunsaturated fatty acids having non-conjugated, carbon-carbon double bonds in the trans configuration., except the Conjugated Linoleic acids (CLA), according to the Afssa's definition (Afssa, 2005) . Until now, only few clinical trials have studied the specific isomeric effects of TFA (IP-TFA *vs.* R-TFA) on CVD.

Recently, two concomitant studies were published. In the first one, 38 healthy men were provided 3 meals/d based on 4 experimental diets: high R-TFA (3.7% of daily energy, ≈ 13.3 g/d), moderate R-TFA (1.5% of daily energy, ≈ 5.6 g/d), high IP-TFA (3.7% of daily energy, ≈ 13.3 g/d), and "control" low total TFA (0.8% of daily energy) for 4 weeks. The consumption of the high IP-TFA and high R-TFA diets had similar consequences, *i.e.* elevated LDL-cholesterol concentrations and decreased HDL-cholesterol levels compared to the consumption of moderate R-TFA or low total TFA diets (Motard-Belanger et al., 2008). The second one is the TransFact study (Chardigny et al., 2008) where 40 healthy subjects consumed food items containing either R-TFA or IP-TFA (11–12 g/d, ≈5% of daily energy intake). Different effects on CVD risk factors are reported according to the 2 sources of TFA but the HDL cholesterol–lowering property of TFA was concluded to be specific to IP-TFA.

Moreover, the consumption for 6 weeks of dairy products naturally enriched in vaccenic acid (the major R-TFA isomer) (around 1.6% daily energy intake) had no effects on most CVD risk parameters in middle-aged men (Tricon et al., 2006). Finally, a 18 y-follow-up study found no association between R-TFA intake and CVD risk factors(Jakobsen et al., 2008).

Modifications of cows' feeding are able to up-regulate the R-TFA content in milk fat with a concomitant reduction in SFA (Chilliard and Ferlay, 2004). These changes in milk fat composition can be considered as a beneficial output (Hu et al., 1997). In that respect during a 5 week intervention study, Tholstrup et al. showed that a butter rich in vaccenic acid (3.6g/d – around 1% daily energy intake) and monounsaturated FAs, significantly decreased total and HDL-cholesterol concentrations in comparison with a conventional butter high in SFA (Tholstrup et al., 2006). From these combined data, the importance of improving R-TFA to SFA ratio in dairy products is suggested. The present study aimed at evaluating in healthy volunteers, the impact on CVD risk factors of milk fats presenting varying ratio between R-TFA and SFA but also MUFA and PUFA. In this respect, a clinical trial where two-thirds of daily fat intake came from experimental dairy fat was designed.

Materials and Methods

Materials. Three experimental dairy fats differing in their fatty acid profiles were obtained from cows fed or not linseed extruded grain or oil; the detailed fatty acid profiles are presented in Table 1. The first one, called "L0" (no linseed supplementation) is the dairy fat with the lowest R-TFA/SFA ratio, i.e. 2.9 and 72 g/100g of fatty acids respectively. The milk was obtained from dairy cows fed a maize silage diet with cereals based concentrate and soybean meal. The second dairy fat, "L4" obtained from cows supplemented with 4.1% on DM basis of extruded linseed (Tradi-Lin® Valorex SAS Combourtillé, France) contained around 4.1 and 63.3 g/100g of R-TFA and SFA, respectively. Finally, "L9" obtained from cows grazing on autumn grass based on a mixture of white clover and perennial rye grass and supplemented with 1 kg of linseed oil (SA huilerie Vandeputte, Mouscron, Belgium) mixed with 5 kg of fresh maize silage. The milk contained around 12.2 and 56.6g /100g of R-TFA and SFA, respectively.

Subjects. Volunteers meeting the following criteria: age 18-50 y, waist circumference < 94 cm for men and < 80 cm for women, HDL-C > 1 mmol/L, LDL-C < 4.1 mmol/L and TG < 1.7 mmol/L were enrolled. The eligibility criteria also included non-smoking, and for women, effective contraception. Characteristics of the volunteers are summarized in Table 2.

Sample size recruitement. The main criterion justifying the number of recruited subjects was the expected L9-induced increase of HDL-cholesterol compared to L0. The difference between L9 and L0 was calculated using the predictive equation of HDL-cholesterol (Yu et al., 1995) and averaged δ=2.17 mg/dL. Sample size (n) was then calculated using the formula $n = (z_\alpha+z_\beta)^2(\sigma/\delta)^2)$ for comparison of two averages (significance level α was chosen to be 5 % two-sided, leading to z_α = 1.96, β was 1-power, and power was set to 80%, leading to z_β = 0.84). According to the TransFact trial (Chardigny et al., 2006) the within subject standard deviation (SD) on this parameter is 4.5 mg/dL. Therefore, 34 subjects per group were needed to detect significant statistical differences

132 ($p<0.05$ two- sided test). To take into account putative drop outs, 37 subjects per group were finally
133 recruited i.e. a total of 111 healthy volunteers (57 men and 54 women).
134
135 ***Human intervention design.*** This study was a controlled, double-blind, randomized trial. It has
136 been approved by the French authorities "Comité Protection des Personnes" (CPP Auvergne,
137 Clermont-Ferrand, France, agreement #AU684). For all subjects, written informed consent was
138 obtained. The Clinical Trial Registration number is NCT00685581. The study design is provided in
139 Figure 1. During the 3 week duration of the intervention, the volunteers consumed three different
140 food items prepared with the 3 experimental fats: butter (20 g/d, 80% fat content), dessert cream
141 (100 g/d, 25% fat content), and cookies (59 g/d, 24% fat content) which corresponded to a total
142 intake of 55 g of lipid (i.e. two-thirds of the total daily lipid intake). Within a day, the experimental
143 products could be consumed during any meal or snack. The three food items were prepared with the
144 three different experimental milk fats (see above). The products were manufactured using the same
145 batch of experimental fat. Microbiological tests and measurement of both total fat and fatty acid
146 (FA) profiles were performed before starting the clinical investigation.
147 During the run-in period (first week, W0), all subjects had to consume L0 food items (Table 1).
148 Thereafter, the volunteers were randomly allocated to one of the three experimental groups after
149 gender stratification was performed. For the following 3 week intervention period, the first group
150 was maintained on the L0 dietary supplementation, whereas the second and the third groups
151 received food items produced from the L4 and the L9 experimental fats, respectively (Figure 1).
152 Fatty acid profile of L9 fat (Table 1) was designed so that the total TFA intake contributed to
153 around 3.1% of daily energy intake (Table 3), which is 2.1% higher than the level recommended by
154 the French authorities (i.e. 2% of TFA excluding CLA of daily energy intake (Afssa, 2005)).
155 The dietician gave instructions to subjects in a documented form to avoid foods containing IP-TFA
156 and ruminant fat. The only source of TFA was the experimental products (R-TFA). All the
157 volunteers were asked to avoid canteens or restaurants during the trial.

Measurements. Subjects attended the laboratory for measurements and blood samples the day after W0 (day 1 of W1) and the day after W3 (day 1 of W4) (Figure 1). Weight was measured at each visit after an overnight fast of at least 12 h, using the same calibrated digital scale with participant dressed in light indoor clothing without shoes. Blood were sampled after an 11h to 15h overnight fast. Plasma was obtained by centrifugation, aliquoted and stored at -80°C until further analyses. The subjects recorded their dietary intake (foods and drinks) during 5 consecutive days, including 3 week days and 2 week end days, during the run-in period (W0) and during the last week of the intervention (W3). Data were coded and analyzed by a dietician using computerized nutrient databases (GENI Micro6.0, Villers-les- Nancy, France).

Biochemical analyses. HDL-cholesterol, total cholesterol, triglycerides, apolipoprotein A1, apolipoprotein B were measured by enzymatic assays using a Konelab 20 analyser (Thermo Electron SA, Cergy-Pontoise, France). LDL-cholesterol concentration was calculated by the Friedewald equation. In order to assess the compliance, plasma phospholipids FA profiles were characterized after plasma lipid extraction and fatty acid methylation. Fatty acid methyl ester profiles were analysed and identified by gas chromatography (Trace GC 2000 Series, ThermoFinnigan, France). The detailed analytical conditions were already reported (Roy et al., 2006). Cholesteryl ester transfer protein (CETP) activity was measured by fluorimetry using commercial kits. Fibrinogen was assessed using a turbidimetric assay (BioDirect, La Villeneuve, France).

Assessment of subjects' compliance. Subject compliance was assessed by a questionnaire and by analysis of the concentration of total *trans*-18:1 and vaccenic acid in plasma phospholipids (Mansour et al., 2001). The mean baseline vaccenic acid concentration in phospholipids was 0.098 ± 0.027 (mean ± standard deviation) g/100 g total fatty acids with no significant effect observed

between groups. At the end of the experimental periods, the average concentrations of vaccenic acid found in plasma phospholipids were 0.160 ± 0.045, 0.252 ± 0.077 and 0.616 ± 0.184 g/100 g total fatty acids for L0, L4 and L9 diet respectively. It was statistically different between the 3 groups (2-way ANOVA, diet: $p<0.0001$, gender $p = 0.489$ interaction $p = 0.473$; post-hoc tests: L0, L4 $p = 0.002$; L0, L9 $p<0.0001$ and L4, L9 $p < 0.0001$).

Statistical Methods. Values are expressed as means± Standard Deviation (SD). Statistical analysis was performed using the Statview version 5.0 software (SAS Institute Inc., Cary, NC). The One way ANOVA procedure was used to determine difference in baseline parameters for the groups. Differences between final and baseline measurements among the three groups were tested by a two-way ANOVA, including diet and gender as factors. If the main effects were significant ($p<0.05$), PLSD Fisher's test was applied for multiple comparisons (post hoc test). We decided to present the results on the per-protocol data set because 3 subjects had already withdrawn during the run-in period before the first measurements (for personal reasons and because of time constraints) and one subject was excluded because he was not compliant. Compliance to the protocol was a primary outcome in the analysis, showing that per-protocol analysis could be performed on the 107 subjects who completed the study (Figure 2).

Results

Dietary intake. During the intervention period, the dietary intake was similar in each experimental group with no gender effect (Table 3). As expected, SFA, PUFA and TFA intake were significantly different between L0, L4, and L9 diets with no gender effects (Table 3).

Plasma lipids, apolipoproteins. Considering the primary outcome i.e. plasma concentrations of HDL-cholesterol, no significant change was evidenced between the three groups. However

210 compared to L0 diet, L4 diet contributed to reduce total cholesterol (p= 0.037), LDL-cholesterol (p
211 = 0.040), LDL-cholesterol/HDL-cholesterol ratio (p = 0.028), and total cholesterol/HDL-cholesterol
212 ratio (p= 0.016), whereas L9 diet did not alter most of these parameters (Table 4).
213 Plasma ApoB concentration tended to be reduced in the L4 group compared to the L0 group, but
214 without reaching the level of significance (p = 0.065).
215 No statistical differences appeared for all the others parameters presented in Table 4.
216

217 **Discussion**

218 The impact of R-TFA on CVD risk markers is a major issue for human nutritional
219 recommendations. Changing the level of R-TFA bio-synthesis in the cows' rumen is associated
220 with a large panel of changes in milk fatty acid content. Our study aimed therefore at examining the
221 metabolic effects of experimental milk fats which represent the widest range of putative milk fatty
222 acid profiles resulting from different cows' feeding strategies. Major finding showed that the
223 consumption of dairy fat containing 63.3% SFA and 3.5% *trans*-18:1 (L4 diet) improved some
224 CVD risk factors for healthy volunteers in comparison with a typical dairy fat (72% SFA, 2.5%
225 *trans*-18:1 –L0 diet). It is illustrated by a decrease in total cholesterol, LDL-cholesterol, total
226 cholesterol/HDL-cholesterol ratio and LDL-cholesterol/HDL-cholesterol ratio. We observed a
227 change by 0.18 units in the ratio of total cholesterol/HDL cholesterol between L0 diet and L4 diet.
228 As reported by Stampfer et al. (Stampfer et al., 1991), we calculate that this change can be
229 associated to a 9.5% decrease in the risk of myocardial infarction, which is in the same range as the
230 replacement of 1334 mg *trans* α-linolenic acid by dietary *cis* α-linolenic (Vermunt et al., 2001).
231 Moreover, our results show that the consumption for 3 weeks of the L9-dairy fat, which contains
232 less SFA (56.6%) and more *trans*-18:1 (9.5%) compared to the L0 diet, induces no significant
233 changes in plasma markers of CVD (Table 4). In addition, the ratio between total and HDL-
234 cholesterol was significantly increased after 3 weeks of L9-dairy fat compared to L4 diet (p =
235 0.029). These data suggest that whereas mild increase in R-TFA/SFA ratio in milk fat may be

236 beneficial compared to L0 diet, further increase in R-TFA/SFA ratio does not provide additional
237 benefit regarding the CVD risk factors.
238 In a study where SFA intake was maintained constant (around 18% of energy intake), a 1.5% total
239 energy intake as R-TFA failed to alter any CVD risk factor (Motard-Belanger et al., 2008).
240 Interestingly in healthy moderately overweight men and women, Rivellese et al. showed that
241 decreasing SFA intake by 8% (from 17.6 to 9.6% total energy intake) and increasing in
242 compensation MUFA intake (from 13.1 to 21.2% total energy intake) induced a reduction in plasma
243 LDL-cholesterol concentration (-0.38 mmol/L) (Rivellese et al., 2003). In our present study, milk
244 fats were characterized by different levels in both R-TFA and SFAs, a higher R-TFA level being
245 associated with a lower SFA content. Notably, high R-TFA/SFA ratio was also associated with
246 enhanced MUFA and PUFA intake. These combined changes in milk fat composition could
247 therefore partially explain the LDL-cholesterol reduction observed after the consumption of the L4
248 diet in comparison with L0 (see Table 4). Our present results are in agreement with the results of
249 Poppit et al. (Poppitt et al., 2002) and Seidel et al.(Seidel et al., 2005). Briefly, Poppit et al.
250 (Poppitt et al., 2002) reported a significant decrease in both total and LDL-cholesterol in plasma
251 from healthy men after consuming a modified butter-fat (-5 units of percent total energy intake of
252 SFA and +2 units of total energy intake of MUFA) for 3 weeks. Seidel et al. (Seidel et al., 2005)
253 demonstrated beneficial effects regarding the CVD risk, i.e. decreased LDL-cholesterol/HDL-
254 cholesterol ratio, with the consumption of modified milk fat obtained by feeding cows high-fat
255 rapeseed cake (16% oil).
256 By contrast, our study shows that the consumption of R-TFA up to 2.42% (L9 diet) of the daily
257 energy intake has no significant effect on the evolution of the HDL concentration which is different
258 from an IP-TFA intake (Katan et al., 1995). However, the differential effect between IP- and R-
259 TFA sources on the HDL parameter seems to disappear for higher TFA intake (3.5% total energy
260 intake) (Motard-Belanger et al., 2008). Even so, our data suggest that whereas mild increase in R-
261 TFA/SFA ratio in milk fat may be beneficial compared to L0 diet, further increase in R-TFA/SFA

ratio does not provide additional benefit regarding the CVD risk factors. Moreover the lack of beneficial effect of the L9 diet could also due to the huge increase in the *trans* 18:2-isomers. These isomers have been reported to be more deleterious than the *trans* 18:1-isomers (Baylin et al., 2003) , for a review see (Mozaffarian and Clarke, 2009)).

During our clinical intervention, we found no significant effect of the consumption of these 3 different diets on the HDL parameter. This result is in accordance with already published trials. Tricon et al. (Tricon et al., 2006) reported that the consumption for 6 weeks of a dairy product naturally enriched in *cis*-9,*trans*-11 CLA (0.2 g/d to 1.5 g/d) and *trans*-11 18:1 (0.8 g/d to 6.3 g/d) failed to alter plasma triacylglycerol, total cholesterol, LDL-cholesterol, and HDL-cholesterol concentrations and total to HDL cholesterol ratio, in healthy middle aged-men. The lack of differences on the HDL parameter could be related to our calculation of the sample size. Indeed, to calculate the sample size, we use the predictive equation of HDL-cholesterol (Yu et al., 1995) and on the other hand we decided that the predicted difference should be δ=2,17 mg/dL: it was perhaps a too small extend in the change in HDL concentrations.

Moreover, our study was carried out in men and women. To our knowledge, there are few studies which assessed the effect of the consumption of modified dairy fat on female CVD risk factors. In our conditions, we found no gender effect, for the relation between the CVD risk factors and fatty acids profiles of dairy fat.

To conclude, we confirm that the consumption of R-TFA at nutritional level (1.01 % L4 diet i.e. <2.0% of energy, the level recommended by the French authorities) have no adverse effect related to some cardiovascular risk factors whatever the gender, which is in accordance with most intervention studies (Motard-Belanger et al., 2008; Seidel et al., 2005) and also with the recent epidemiological study (Jakobsen et al., 2008). Moreover, this clinical study underlines the fact that, cows' feeding strategy consisting in decreasing the SFA/TFA ratio (less SFA (56.6%) and more

total *trans* (12.16 %)) in fat does not bring any additional benefits regarding the CVD risk in healthy volunteers.

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Literature Cited

Afssa (2005): Risques et bénéfices pour la santé des acides gras trans apportés par les aliments- Health risks and benefits of trans fatty acids in food - Recommendations (synthesis), pp. 1-217, AFSSA, Maison-Alfort.

Ascherio A, Katan MB, Zock PL, Stampfer MJ and Willett WC (1999): Trans fatty acids and coronary heart disease. *N Engl J Med*. **340**, 1994-8.

Baylin A, Kabagambe EK, Ascherio A, Spiegelman D and Campos H (2003): High 18:2 trans-fatty acids in adipose tissue are associated with increased risk of nonfatal acute myocardial infarction in costa rican adults. *J Nutr*. **133**, 1186-91.

Booker CS and Mann JI (2008): Trans fatty acids and cardiovascular health: translation of the evidence base. *Nutr Metab Cardiovasc Dis*. **18**, 448-56.

Chardigny JM, Destaillats F, Malpuech-Brugere C, Moulin J, Bauman DE, Lock AL, Barbano DM, Mensink RP, Bezelgues JB, Chaumont P, Combe N, Cristiani I, Joffre F, German JB, Dionisi F, Boirie Y and Sebedio JL (2008): Do trans fatty acids from industrially produced sources and from natural sources have the same effect on cardiovascular disease risk factors in healthy subjects? Results of the trans Fatty Acids Collaboration (TRANSFACT) study. *Am J Clin Nutr*. **87**, 558-66.

Chardigny JM, Malpuech-Brugere C, Dionisi F, Bauman DE, German B, Mensink RP, Combe N, Chaumont P, Barbano DM, Enjalbert F, Bezelgues JB, Cristiani I, Moulin J, Boirie Y, Golay PA, Giuffrida F, Sebedio JL and Destaillats F (2006): Rationale and design of the TRANSFACT project phase I: A study to assess the effect of the two different dietary

sources of trans fatty acids on cardiovascular risk factors in humans. *Contemp Clin Trials*. **27**, 364-73.
Chilliard Y and Ferlay A (2004): Dietary lipids and forages interactions on cow and goat milk fatty acid composition and sensory properties. *Reprod Nutr Dev*. **44**, 467-92.
Dalainas I and Ioannou HP (2008): The role of trans fatty acids in atherosclerosis, cardiovascular disease and infant development. *Int Angiol*. **27**, 146-56.
European Heart Network (2008): European cardiovascular disease statistics, pp. 7-8. In Allender S., Scarborough P., Peto V., Rayner M., Leal J., Luengo-Fernandez R., et al. (Eds): *2008 Edition*, European Heart Network.
Gebauer SK, Psota TL, Harris WS and Kris-Etherton PM (2006): n-3 fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits. *Am J Clin Nutr*. **83**, 1526S-1535S.
Gebauer SK, Psota TL and Kris-Etherton PM (2007): The Diversity of Health Effects of Individual trans Fatty Acid Isomers. *Lipids*. **42**, 787-99.
Hu FB, Stampfer MJ, Manson JE, Rimm E, Colditz GA, Rosner BA, Hennekens CH and Willett WC (1997): Dietary fat intake and the risk of coronary heart disease in women. *N Engl J Med*. **337**, 1491-9.
Jakobsen MU, Overvad K, Dyerberg J and Heitmann BL (2008): Intake of ruminant trans fatty acids and risk of coronary heart disease. *Int J Epidemiol*.
Katan MB, Zock PL and Mensink RP (1995): Trans fatty acids and their effects on lipoproteins in humans. *Annu Rev Nutr*. **15**, 473-93.
Mansour MP, Li D and Sinclair AJ (2001): The occurrence of trans-18:1 isomers in plasma lipids classes in humans. *Eur J Clin Nutr*. **55**, 59-64.
Mensink RP, Zock PL, Kester AD and Katan MB (2003): Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. *Am J Clin Nutr*. **77**, 1146-55.
Motard-Belanger A, Charest A, Grenier G, Paquin P, Chouinard Y, Lemieux S, Couture P and Lamarche B (2008): Study of the effect of trans fatty acids from ruminants on blood lipids and other risk factors for cardiovascular disease. *Am J Clin Nutr*. **87**, 593-9.
Mozaffarian D and Clarke R (2009): Quantitative effects on cardiovascular risk factors and coronary heart disease risk of replacing partially hydrogenated vegetable oils with other fats and oils. *Eur J Clin Nutr*. **63 Suppl 2**, S22-33.
Poppitt SD, Keogh GF, Mulvey TB, McArdle BH, MacGibbon AK and Cooper GJ (2002): Lipid-lowering effects of a modified butter-fat: a controlled intervention trial in healthy men. *Eur J Clin Nutr*. **56**, 64-71.
Rivellese AA, Maffettone A, Vessby B, Uusitupa M, Hermansen K, Berglund L, Louheranta A, Meyer BJ and Riccardi G (2003): Effects of dietary saturated, monounsaturated and n-3 fatty acids on fasting lipoproteins, LDL size and post-prandial lipid metabolism in healthy subjects. *Atherosclerosis*. **167**, 149-58.
Roy A, Ferlay A and Chilliard Y (2006): Production of butter fat rich in trans10-C18:1 for use in biomedical studies in rodents. *Reprod Nutr Dev*. **46**, 211-8.
Scientific Panel on Dietetic Products NaA (2004): Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to the presence of trans fatty acids in foods and the effect on human health of the consumption of trans fatty acids (Request N° EFSA-Q-2003-022), pp. 1-49: *The EFSA Journal* EFSA.
Seidel C, Deufel T and Jahreis G (2005): Effects of fat-modified dairy products on blood lipids in humans in comparison with other fats. *Ann Nutr Metab*. **49**, 42-8.
Stampfer MJ, Sacks FM, Salvini S, Willett WC and Hennekens CH (1991): A prospective study of cholesterol, apolipoproteins, and the risk of myocardial infarction. *N Engl J Med*. **325**, 373-81.

Stender S and Dyerberg J (2003): The influence of trans fatty acids on health. 4th ed. A report from The Danish Nutrition Council Publication. *The Danish Nutrition Council*. **Publication No. 34, Copenhagen**.

Stender S and Dyerberg J (2004): Influence of trans fatty acids on health. *Ann Nutr Metab*. **48**, 61-6.

Tholstrup T, Raff M, Basu S, Nonboe P, Sejrsen K and Straarup EM (2006): Effects of butter high in ruminant trans and monounsaturated fatty acids on lipoproteins, incorporation of fatty acids into lipid classes, plasma C-reactive protein, oxidative stress, hemostatic variables, and insulin in healthy young men. *Am J Clin Nutr*. **83**, 237-43.

Tricon S, Burdge GC, Jones EL, Russell JJ, El-Khazen S, Moretti E, Hall WL, Gerry AB, Leake DS, Grimble RF, Williams CM, Calder PC and Yaqoob P (2006): Effects of dairy products naturally enriched with cis-9,trans-11 conjugated linoleic acid on the blood lipid profile in healthy middle-aged men. *Am J Clin Nutr*. **83**, 744-53.

Vermunt SH, Beaufrere B, Riemersma RA, Sebedio JL, Chardigny JM, Mensink RP and TransLin EIa (2001): Dietary trans alpha-linolenic acid from deodorised rapeseed oil and plasma lipids and lipoproteins in healthy men: the TransLinE Study. *Br J Nutr*. **85**, 387-92.

Yu S, Derr J, Etherton TD and Kris-Etherton PM (1995): Plasma cholesterol-predictive equations demonstrate that stearic acid is neutral and monounsaturated fatty acids are hypocholesterolemic. *Am J Clin Nutr*. **61**, 1129-39.

Figure legends

Figure 1 Study design. (Dietary questionnaire)

Figure 2 Disposition of subjects (*n=126*) during the study.

Table 1 Fatty acid composition of the different experimental dairy fats (g fatty acid/100g of fatty acids)

Fatty acids	**Fatty acid composition**		
	L0[b]	**L4**[b]	**L9**[b]
C4:0	2.54	2.83	2.94
C5:0	0.04	0.03	0.03
C6:0	1.80	1.76	1.95
C7:0	0.03	0.02	0.02
C8:0	1.20	1.06	1.24
C9:0	0.03	0.03	0.03
C10:0	3.09	2.33	2.86
C10:1	0.30	0.19	0.27
C11:0	0.07	0.04	0.05
C12:0	3.95	2.88	3.31
C13:0	0.22	0.13	0.17
C14:0	12.84	9.75	11.04
C14:1	1.00	0.64	0.94
C15:0	1.26	1.08	0.94
C16:0	34.60	27.94	21.93
C16:1	1.55	1.65	1.02
C17:0	0.73	0.79	0.53
C17:1	0.21	0.35	0.15
C18:0	9.41	12.42	9.43
C18:1 *trans* Total	2.53	3.49	9.50
trans-4	0.01	0.01	0.04
trans-5	0.01	0.01	0.03
trans-6/8	0.21	0.21	0.58
trans-9	0.21	0.23	0.45
trans-10	0.29	0.33	1.23
trans-11	1.00	1.81	4.26
trans-12	0.29	0.29	0.86
trans-13	0.51	0.59	2.04
***trans*-9+*trans*-10+*trans*-11**	1.51	2.37	5.94
C18:1 n-9	15.53	21.87	17.12
C18:1 *cis*-14+*trans*-16	0.28	0.35	0.63
C18:1 *cis*-15+C19:0	0.16	0.21	0.53
Other *cis*-C18:1 isomers	0.94	0.97	1.49
trans-C18:2	0.32	0.57	2.66
CLA	0.42	0.67	1.86
C18:2 n-6	1.34	1.31	2.06
C18:3 n-3	0.22	0.59	1.22
C20:0	0.11	0.13	0.07
C20:2 n-6	0.01	0.01	0.02
C20:3 n-6	0.06	0.04	0.05
C20:4 n-6	0.09	0.07	0.08
C20:5 n-3	0.03	0.08	0.04
C22:0	0.04	0.05	0.02
C24:0	0.02	0.03	0.02
C22:5	0.05	0.09	0.05
Other fatty acids	3.00	3.53	3.72
Sum (12:0+14:0+16:0)	51.39	40.57	36.27
Total saturated fatty acids	71.97	63.31	56.59
Total cis-MUFA	19.52	25.66	20.99
Total trans fatty acids[a]	2.85	4.06	12.16
Total cis-PUFA	2.21	2.87	5.37

[a]Sum of trans-18:1 and trans-18:2 acid isomers; Conjugated Linoleic Acid (CLA) are not taken into account in this calculation; [b]L0, L4, L9 see Method section, cis-MUFA: cis-monounsaturated fatty acids; cis-PUFA: cis-polyunsaturated fatty acids

Table 2 Baseline characteristics (by study group) of subjects who completed the trial.

Parameter	L0 group	L4 group	L9 group	p-Value
Clinical				
Gender* (M/F)	18/18	18/17	18/18	0.990
Age (y)	26 ±7 [12; 40]	25 ±6 [13; 37]	28 ±9 [10; 45]	0.394
Waist (cm)	74.1±9.0 [56.6; 91.7]	74.4±8.1 [58.6; 90.2]	71.3±8.1 [55.3; 87.2]	0.997
Body mass index (kg/m^2)	21.7±2.7 [16.5; 26.9]	22.0±2.3 [17.5; 26.5]	21.9±2.5 [16.9; 26.8]	0.891
Systolic blood pressure (mm Hg)	116±9 [97; 134]	116±8 [100; 132]	116±13 [91; 141]	0.997
Diastolic blood pressure (mm Hg)	73±8 [58; 88]	71±9 [53; 89]	72±9 [54; 90]	0.654
Resting heart rate (beat per min)	67±8 [51; 83]	64±7 [50; 78]	68±10 [48; 88]	0.122
Glucose (mmol/L)	4.6±03 [4.0; 5.3]	4.6±0.4 [3.8; 5.5]	4.7±0.5 [3.7; 5.6]	0.919
Bilirubin (µmol/L)	14±9 [-3; 32]	13±8 [-2; 29]	13±6 [1; 25]	0.709
ASAT (UI/L)	23±5 [13; 32]	23±4 [14; 31]	23±5 [12; 33]	0.927
ALAT (UI/L)	17±8 [1; 33]	18±9 [1; 35]	17±7 [3; 30]	0.685
Phosphatase alkaline (UI/L)	58±14 [31; 85]	59±21 [18; 100]	56±13 [30; 81]	0.653
γ-Glutamyl transpeptidase (UI/L)	14±7 [0; 28]	18±12 [-6; 42]	15±7 [0; 29]	0.135
Na (mmol/L)	142±2 [138; 145]	141±2 [138; 145]	141±2 [138; 145]	0.572
K (mmol/L)	4.3±0.3 [3.6; 4.9]	4.2±0.3 [3.7; 4.8]	4.2±0.3 [3.6; 4.8]	0.687
Cl (mmol/L)	103±2 [100; 106]	103±2 [100; 106]	103±1 [100; 106]	0.919
Urea (mmol/L)	5.1±1.2 [2.8; 7.3]	5.3±1.5 [2.4; 8.3]	4.9±1.3 [2.4; 7.5]	0.429
Creatinin (µmol/L)	75±10 [56; 94]	78±11 [56; 100]	75±12 [52; 97]	0.354
Erythrocytes (T/L)	4.87±0.38 [4.13; 5.61]	4.82±0.36 [4.11; 5.53]	4.79±0.41 [3.99; 5.58]	0.621
Haemoglobin (g/dL)	14.3±1.2 [12.0; 16.6]	14.0±1.1 [12.0; 16.1]	14.0±1.2 [11.8; 16.3]	0.525
Haematocrit (%)	42.3±3.1 [36.1; 48.4]	41.6±2.5 [36.8; 46.4]	41.6±3.0 [35.8; 47.4]	0.530
Mean Globular Volume (fL)	86.8±2.5 [81.9; 91.7]	86.4±3.0 [80.6; 92.2]	87.1±3.8 [79.7; 94.5]	0.624
Platelets (G/L)	224±38[a] [148; 299]	255±42[b] [172; 337]	247±52[b] [146; 349]	0.01
Leukocytes (G/L)	5.96±1.36 [3.29; 8.63]	6.25±1.55 [3.22; 9.28]	5.68±1.28 [3.16; 8.19]	0.234
Neutrophils (G/L)	3.12±1.17 [0.83; 5.40]	3.30±1.14 [1.07; 5.54]	2.98±0.90 [1.22; 4.74]	0.446
Eosinophils (G/L)	0.16±0.10 [0.03; 0.35]	0.16±0.10 [0.03; 0.35]	0.17±0.16 [0.15; 0.49]	0.839
Basophils (G/L)	0.02±0.02 [0.01; 0.06]	0.02±0.01 [0.00; 0.05]	0.03±0.01 [0.00; 0.05]	0.587
Lymphocytes (G/L)	2.14±0.58 [1.00; 3.28]	2.23±0.73 [0.79; 3.66]	2.03±0.65 [0.76; 3.30]	0.454
Monocytes (G/L)	0.52±0.13 [0.27; 0.77]	0.53±0.18 [0.19; 0.87]	0.48±0.13 [0.22; 0.74]	0.337
Fasting chemical lipids				
HDL-C (mmol/L)	1.69±0.33 [1.03; 2.34]	1.76±0.50 [0.79; 2.74]	1.62±0.40 [0.84; 2.39]	0.348
LDL-C (mmol/L)	2.34±0.67 [1.02; 3.66]	2.46±0.75 [0.99; 3.93]	2.35±0.79 [0.80; 3.91]	0.760
Triacylglycerol (mmol/L)	0.81±0.25 [0.31; 1.30]	0.85±0.32 [0.23;1.47]	0.69±0.28 [0.13; 1.25]	0.052
Cholesterol (mmol/L)	4.39±0.69 [3.05; 5.74]	4.61±0.82 [2.99; 6.22]	4.29±0.86 [2.59; 5.98]	0.226

Values are expressed as mean ± SD and 95% confidence intervals [95% CIs]* Number of male and females, respectively. Data were analyzed by a one way ANOVA.

Table 3 Mean daily intake and 95% confidence intervals [95% CIs] of energy and macronutrients in L0, L4 and L9 groups, at baseline and after the 3 week intervention period (follow-up).

	L0 group (n = 36)		L4 group (n = 35)		L9 group (n = 36)		ANOVA		
	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	Gender	Diet	Gender x Diet
	Mean ±SD [95% CIs]						p	p	p
Total Energy, kJ/d	8610±1404	8782±1602	8556±1884	8583±1486	8104±1539	8375±1519	0.225	0.670	0.655
	[-5858; 11361]	[-5642; 11923]	[-4864; 12249]	[-5671; 11495]	[-5086; 1121]	[-5398; 11352]			
Protein,	14.8±3.0	14.9±2.9	15.0±2.7	15.4±2.7	14.1±2.8	14.5±2.4	0.284	0.666	0.968
%en	[-9.0; 20.6]	[-9.3 20.5]	[-9.7; 20.3]	[-10.2; 20.7]	[-8.6; 19.6]	[-9.8; 19.2]			
Carbohydrate, %en	47.5±5.9	47.4±5.9	45.9±5.3	44.9±5.0	47.2±5.7	46.7±4.9	0.453	0.912	0.485
	[-36.0; 59.0]	[-35.9; 59.0]	[-35.5; 56.2]	[-36.0; 55.7]	[-35.9; 58.4]	[-37.2; 56.2]			
Total Fat,	37.7±5.4	37.7±5.0	39.2±5.0	38.7±5.1	38.8±5.4	38.8±4.8	0.805	0.876	0.612
%en	[-27.1; 48.2]	[-27.8; 47.5]	[-29.4; 49.0]	[-28.8; 48.6]	[-28.3; 49.3]	[-29.4; 48.2]			
SFA,	21.5±2.6	21.3±2.8[a]	22.1±3.0	19.9±2.9[b]	22.6±3.3	18.1±2.4[c]	0.308	<0.0001	0.965
%en	[-16.4;26.6]	[-15.8; 26.8]	[-16.2; 28.0]	[-14.2; 25.5]	[-16.1; 29.0]	[-13.4; 22.9]			
MUFA,	11.4±2.7	11.8±2.6[a]	11.7±2.5	14.0±2.6[b]	11.9±2.6	14.3±2.2[b]	0.607	0.0003	0.904
%en	[-6.0; 16.7]	[-6.7; 16.8]	[-6.9; 16.5]	[-9.0; 19.1]	[-6.7; 17.0]	[-10.1; 18.6]			
PUFA,	3.6±1.3	3.6±1.1[a]	3.5±1.2	3.9±1.3[b]	3.6±1.2	5.2±1.0[c]	0.380	<0.0001	0.531
%en	[-1.1; 6.1]	[-1.4; 5.7]	[-1.1; 5.9]	[-1.3; 6.4]	[-1.3; 5.9]	[-3.2; 7.1]			
Total TFA*	0.70±0.11	0.69±0.11[a]	0.72±0.14	1.01±0.18[b]	0.75±0.13	3.10±0.55[c]	0.169	<0.0001	0.148
%en	[-0.49; 0.91]	[-0.47 ; 0.91]	[-0.45; 0.98]	[-0.65;1.36]	[-0.49; 1.01]	[-2.02; 4.18]			
Total trans-18:1*	0.62±0.10	0.61±0.10[a]	0.64±0.12	0.87±0.16[b]	0.67±0.12	2.42±0.43[c]	0.236	<0.0001	0.173
%en	[-0.43; 0.81]	[-0.43 ; 0.81]	[-0.40; 0.87]	[-0.56; 1.17]	[-0.44; 0.90]	[-1.57; 3.27]			

All values are means ± SD. Data (the difference between end of the intervention and baseline) were analyzed using a 2-way ANOVA with gender and diet as factors.

Means in a row without common superscript letters differ.

%en: % of total energy, SFA, saturated fatty acids; cis-MUFA: cis-monounsaturated fatty acids; cis-PUFA: cis-polyunsaturated fatty acids; TFA, *trans* fatty acids. *: this represents only the percentage of TFA and total trans-18:1 in the three different food items (butter, dessert cream, and cookies).

Table 4 Serum lipids, lipoprotein, apolipoprotein concentrations, cholesterol ester transfer protein (CETP) activity and fibrinogen concentration in the three different groups (L0, L4 and L9 group) mean and 95% confidence intervals [95% CIs] at baseline and estimate mean effects after 3 weeks.

Variable and subjects	Baseline values[1]			Estimate mean effects[2]			p-Value		
	L0 group (n = 36)	**L4 group** (n = 35)	**L9 group** (n = 36)	**L0**	**L4**	**L9**	**Diet**	**Gender**	**Interaction**
HDL-C (mmoL/L)	1.70 ± 0.44 [0.85; 2.56]	1.74 ± 0.51 [0.74; 2.74]	1.59±0.32 [0.97; 2.21]	0.01±0.16 [-0.31; 0.33]	0.05±0.17 [-0.29; 0.39]	0.00±0.15 [-0.30; 0.29]	0.378	0.457	0.965
LDL-C (mmoL/L)	2.33 ± 0.77 [0.82; 3.83]	2.65 ± 0.83 [1.02; 4.28]	2.55 ± 0.90 [0.78; 4.32]	0.11±0.33[a] [-0.53; 0.75]	-0.14±0.38[b] [-0.72; 0.77]	-0.07±0.42[a,b] [-0.89; 0.76]	0.040	0.759	0.386
Total cholesterol (mmol/L)	4.42 ± 0.78 [2.88; 5.95]	4.88 ± 0.86 [3.19; 6.57]	4.52 ± 0.93 [2.70; 6.34]	0.1±0.42[a] [-0.68; 0.95]	-0.13±0.50[b] [-1.11; 0.85]	-0.05±0.42[a, b] [-0.87; 0.77]	0.037	0.448	0.332
TG (mmol/L)	0.85 ± 0.31 [0.24; 1.47]	1.08 ± 0.53 [0.04; 2.12]	0.82 ± 0.29 [0.25; 1.40]	0.05 ± 0.27 [-0.48; 0.57]	-0.10 ± 0.46 [-0.99; 0.80]	0.04±0.35 [-0.64; 0.72]	0.198	0.629	0.094
ApoA1 (g/L)	1.52 ± 0.25 [1.04; 2.01]	1.63 ± 0.33 [0.98; 2.29]	1.48 ± 0.20 [1.09; 1.88]	0.04±0.13 [-0.21; 0.29]	0.01±0.11 [-0.20; 0.22]	0.00±0.08 [-0.16; 0.16]	0.387	0.980	0.168
ApoB (g/L)	0.79 ± 0.19 [0.42; 1.16]	0.88 ± 0.21 [0.47; 1.28]	0.81 ± 0.22 [0.37; 1.24]	0.02 ± 0.09 [-0.15; 0.20]	-0.03± 0.10 [-0.22; 0.16]	0.01± 0.12 [-0.22; 0.24]	0.065	0.840	0.221
LDL-C/HDL-C	1.47 ± 0.65 [0.21; 2.74]	1.69 ± 0.70 [0.31; 3.06]	1.68 ± 0.73 [0.25; 3.12]	0.06 ± 0.22[a] [-0.37; 0.50]	-0.14 ± 0.36[b] [-0.84; 0.57]	0.00 ± 0.33[a,b] [-0.66; 0.65]	0.028	0.837	0.587
Total cholesterol/HDL-C	2.73 ± 0.74 [1.27; 4.18]	3.00 ± 0.85 [1.33; 4.68]	2.93 ± 0.79 [1.39; 4.47]	0.07 ± 0.28[a] [-0.47; 0.61]	-0.18 ± 0.44[b] [-1.05; 0.68]	0.01 ± 0.39[a] [-0.74; 0.77]	0.016	0.761	0.293
ApoB/ApoA1	0.53 ± 0.15 [0.23; 0.84]	0.56 ± 0.16 [0.25; 0.87]	0.55 ± 0.17 [0.23; 0.87]	0.00 ± 0.06 [-0.12; 0.12]	-0.03 ± 0.07 [-0.16; 0.11]	0.01 ± 0.08 [-0.14; 0.16]]	0.133	0.782	0.577
CETP activity (nmol/h/mL)	16.87 ± 3.97 [9.10; 24.66]	17.04 ± 4.66 [7.91; 26.18]	18.12 ± 4.30 [9.69; 26.56]	0.23 ± 6.83 [-13.15; 13.61]	0.61 ± 8.39 [-15.84; 17.07]	0.03 ± 7.31 [-14.29; 14.36]	0.944	0.630	0.701
Fibrinogen (g/L)	2.68 ± 0.53 [1.64; 3.72]	2.75 ± 0.57 [1.64; 3.86]	2.70 ± 0.53 [1.65; 3.74]	-0.56 ± 0.52 [-1.58; 0.46]	-0.49 ± 0.43 [-1.34; 0.36]	-0.50 ± 0.55 [-1.59; 0.58]	0.843	0.458	0.744

[1]means ± SD, [2] Estimate mean effects is defined as the difference between end of the intervention and baseline. Data are analyzed by using a two-ways anova. Means in a row without common superscript letters differ.

Abbreviations

ALT: Alanine aminotransférase

AST: Aspartate aminotransférase

βHCG: Human chorionic gonadotropin

CETP: Cholesteryl ester transfer protein

CPP: Comité de protection des personnes

CRNH: Centre de recherche en nutrition humaine

CRP: C reactive protein

CVD: Cardiovascular disease

DQ: Dietary Questionnaire

EU: European Union

γGT: Gamma glutamyltransférase

HCV: Hepatitis C virus

IP-TFA: Industrially produced *trans* fatty acids

PHVO: Partially hydrogenated vegetable oils

R-TFA: Ruminant *trans* fatty acids

TFA: *Trans* fatty acids

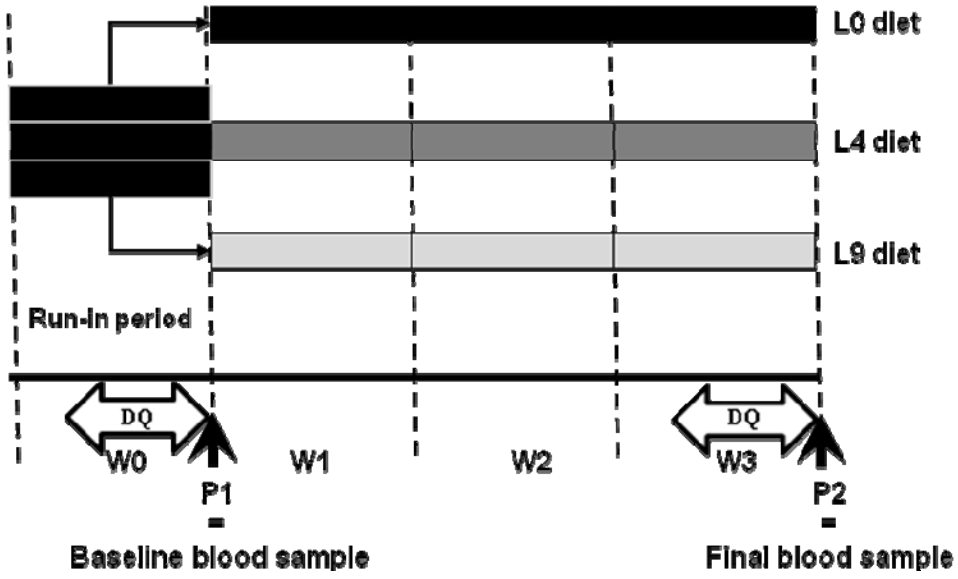
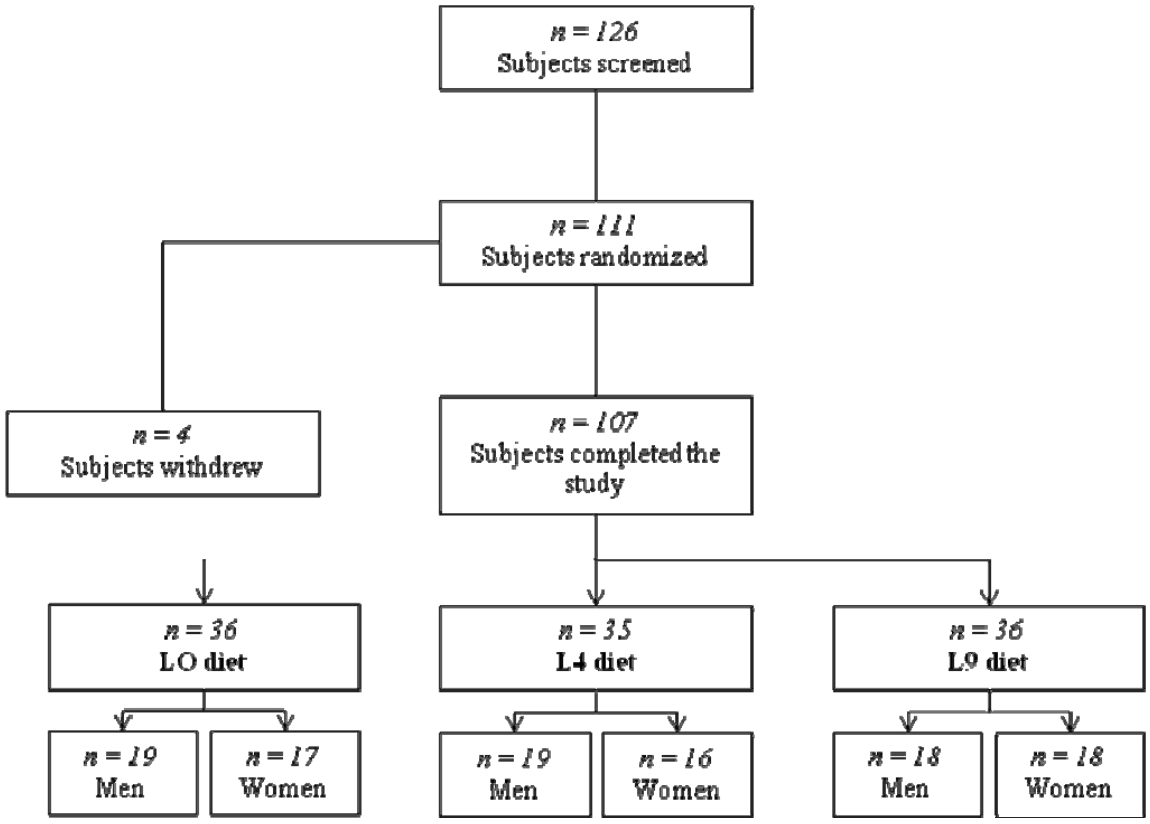

L0 diet
L4 diet
L9 diet
Run-in period
DQ
W0
W1
W2
DQ
W3
P1
P2
Baseline blood sample
Final blood sample

Figure 1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Subjects screened
n = 111
Subjects randomized
n = 4
Subjects withdrew
n = 107
Subjects completed the study
n = 36
LO diet
n = 35
L4 diet
n = 36
L9 diet
n = 19
Men
n = 17
Women
n = 19
Men
n = 16
Women
n = 18
Men
n = 18
Women

Figure 2